Invasive Infection Caused by Community-Associated Methicillin-Resistant

*Staphylococcus aureus* Strain Not Carrying Panton-Valentine Leukocidin in

Korea

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**Running title:** PVL-negative CA-MRSA invasive infection

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Panton-Valentine leukocidin (PVL)-negative, SCCmec type IVa strains are the most common strains of methicillin-resistant Staphylococcus aureus (MRSA) circulating in the community in Korea. This report describes five elderly patients presenting in 2006–2007 with invasive community associated-MRSA infection caused by PVL-negative, SCCmec type IVa strain with sequence type 72 and spa type t324.
Panton-Valentine leukocidin (PVL) genes are prevalent among community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) strains and has been known as one of the key virulence determinants of CA-MRSA strains (18, 22). Recently, however, some studies have suggested that PVL genes are differentially distributed among CA-MRSA strains, and multiple factors, rather than any single determinant such as PVL, promotes CA-MRSA infection (15, 19). PVL-negative, CA-MRSA strains are predominant in Asian-Pacific countries such as Australia, Japan, and China as well as Korea (1, 3, 7, 17, 21). Especially, a new clone of CA-MRSA, sequence type (ST) 72-SCCmec type IVa, which lacks PVL genes, is the most predominant clonal type in Korea (7, 14). Although there have been some epidemiologic studies of CA-MRSA, few reports are available for invasive infection caused by these PVL-negative CA-MRSA strains in Korea. We present five Korean cases of invasive CA-MRSA infection caused by PVL-negative, SCCmec type IVa strain with sequence type 72 and spa type t324. **Patient 1.** An 80-year-old woman with type 2 diabetes mellitus (DM) suddenly developed painful swelling of the left proximal forearm and right knee. With a clinical diagnosis of infectious bursitis, she was initially treated with intravenous cefazolin after incision and drainage. The antibiotic regimen was changed to intravenous vancomycin since pus culture obtained at admission yielded MRSA. However, the infection
progressed rapidly to necrotizing fasciitis necessitating an emergency fasciotomy. After a 6-week course of intravenous vancomycin therapy, she was ultimately discharged without sequelae.

**Patient 2.** A 66-year-old woman with type 2 DM presented with painful swelling of the left buttock and was initially treated with intravenous cefazolin. On hospital day 3, the lesion spread rapidly to the left thigh and perineum, and septic shock with acute renal failure developed. Blood cultures obtained at the emergency department yielded MRSA. She was started on intravenous teicoplanin and piperacillin/tazobactam followed by an emergency fasciotomy with a diagnosis of necrotizing fasciitis. On hospital day 5, she was transferred to other tertiary-care hospital, whereupon it was learned that blood cultures taken at our hospital were positive for MRSA. Ultimately, she died of an uncontrolled infection.

**Patient 3.** A 68-year-old man with chronic obstructive lung disease was admitted with a 2-day history of fever and purulent sputum. A chest computed tomography revealed necrotizing pneumonia and multiple lung abscess in the right lower lung field, the largest of which was 7.7 cm × 2.8 cm. Treatment with piperacillin/tazobactam and ciprofloxacin was initiated. His antibiotic regimen was changed to vancomycin after cultures of sputum samples obtained on hospital day 2 yielded MRSA. On hospital day
8, empyema developed and a chest tube was inserted. With a 4-weeks course of intravenous vancomycin, the patient was cured without sequelae.

**Patient 4.** A 71-year-old woman with a history of mitral valve stenosis and atrial fibrillation developed fever one day before admission. Chest examination revealed a grade 3 systolic murmur at apex. Janeway lesions were observed on both feet. A 1-cm vegetation in the left atrium was observed on transthoracic echocardiography. Despite administration of intravenous cefazolin and gentamicin with a diagnosis of acute infective endocarditis, septic shock developed. Blood cultures obtained at admission yielded MRSA and cefazolin was changed to vancomycin. With the administration of intravenous vancomycin for 6 weeks, she was ultimately discharged without sequelae.

**Patient 5.** A 82-year-old man presented with right lower leg pain, which had gradually worsened during the previous 6 months. On the right lateral malleolus, pus-like discharge was drained. Magnetic resonance imaging demonstrated a gadolinium-enhanced lesion consistent with osteomyelitis. Cultures of pus obtained at admission subsequently grew MRSA. The patient received a total of 6 weeks of intravenous vancomycin after incision and drainage.

All five elderly patients with invasive CA-MRSA infection were identified at Hallym University Sacred Heart Hospital from 2006 to 2007. Community acquisition of MRSA
was defined as the growth of isolates either in outpatient setting or within 48 h of
hospital admission in patients who had no risk factors for MRSA acquisition (past
medical history of MRSA infection or colonization; history of admission to a hospital or
long-term care facilities during the past year; history of surgery, dialysis, permanent
indwelling catheters or medical devices that pass through the skin to the body during the
past year) (5).

*S. aureus* strains were identified by MicroScan (Dade Behring, West Sacramento, CA)
and conventional methods such as coagulase test, mannitol fermentation, and DNase
test. Antimicrobial susceptibility testing was done using the MicroScan and disk
diffusion methods. Resistance to methicillin was determined by the oxacillin disk
susceptibility test and the presence of the *mecA* gene (10, 12). SCC*mec* typing was
performed using multiplex polymerase chain reaction (PCR) (11, 12). Multilocus
sequence typing (MLST) was performed as previously reported (4), as was *spa* typing
(16). The *spa* types were determined by comparing the database at the Ridom SpaServer
website (http://www.ridom.de/spa-server/). PCR amplification for staphylococcal
enterotoxin genes (*sea, seb, sec, sed, see*), toxic shock syndrome toxin gene (*tst*),
exfoliative toxin genes (*eta* and *etb*), and PVL genes were performed as previously
reported (8, 10).
All five of the presently reported elderly patients had no risk factors for MRSA acquisition, and culture of all specimens was performed within 48 h of hospital admission. Antibiogram patterns were identical in all CA-MRSA isolates, in which MRSA isolates were susceptible to all non-β-lactam antibiotics except erythromycin and clindamycin (Table). These five isolates were susceptible to clindamycin by disk diffusion testing, but D-test positive. All five MRSA isolates harbored the SCCmec type IVa element (12). Also, these five MRSA isolates were classified as type IV SCCmec, when they were tested by use of the updated SCCmec multiplex PCR assay described in 2007 (11). MLST and spa typing revealed sequence type 72 and spa type t324 in all CA-MRSA isolates. None of the isolates possessed the PVL gene and other staphylococcal toxin genes tested. These ST 72-SCCmec IVa-spa type t324 strains have been reported in children adopted to Europe from Korea, and most cases of transmission of MRSA from these children to other family members were spa type t324 (6). In addition, ST 72-SCCmec IV strains with spa type t2431, a closely related type of spa type t324, has been reported in Portugal (1). CA-MRSA strains share the SCCmec type IV element and increased susceptibilities to non-β-lactam antibiotics, but have multiple genetic backgrounds. PVL genes are present among the prototype CA-MRSA strain MW2 and related strains (spa types t131 and
t194), but are differentially distributed among strains of *spa* types t1, t7, t17, and other miscellaneous *spa* types (15). Considering the result of *spa* typing of CA-MRSA isolates found in Korea, in which the most prevalent *spa* type was *spa* type t324 (14), it is possible that PVL genes might not be detected among CA-MRSA isolates in Korea. Recently, a case of perianal abscess caused by PVL-positive CA-MRSA strain, USA300 and *spa* type t1 in Korea has been reported, but the organism might have been imported from Hawaii, because the patient had been to Hawaii a few weeks before MRSA infection (13). Also, it has been suggested that PVL is not the key virulent determinant of CA-MRSA (2, 15, 19). In our study, none of invasive CA-MRSA isolates possessed staphylococcal enterotoxin genes and toxic shock syndrome toxin gene as well as PVL genes. Further studies about other virulence factors of CA-MRSA, such as phenol-soluble modulins (PSMs) and α-hemolysin will be required (2, 9, 20). Although most CA-MRSA isolates were found in patients with underlying diseases, and host factors might contribute to severe MRSA infection irrespective of the presence of the PVL gene, presently PVL-negative CA-MRSA isolates with ST72 and *spa* type t324 caused invasive infections including necrotizing pneumonia and fatal necrotizing fasciitis in elderly patients.
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Table. Molecular characteristics and antimicrobial susceptibility of CA-MRSA isolates.

<table>
<thead>
<tr>
<th>Age/Sex</th>
<th>Source of MRSA</th>
<th>SCC_mec sequence type</th>
<th>spa type</th>
<th>CA</th>
<th>CL</th>
<th>CIP</th>
<th>EM</th>
<th>GM</th>
<th>RFP</th>
<th>SMX</th>
<th>TET</th>
<th>VM</th>
</tr>
</thead>
<tbody>
<tr>
<td>80/Male</td>
<td>pus</td>
<td>- IVa</td>
<td>ST72 t324</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>66/Female</td>
<td>pus, blood</td>
<td>- IVa</td>
<td>ST72 t324</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>68/Male</td>
<td>sputum</td>
<td>- IVa</td>
<td>ST72 t324</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>71/Female</td>
<td>blood</td>
<td>- IVa</td>
<td>ST72 t324</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>82/Male</td>
<td>pus</td>
<td>- IVa</td>
<td>ST72 t324</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
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</table>

Note. * These strains showed positive D-test result but clindamycin-susceptible by single-disk testing.

CA-MRSA, methicillin-resistant Staphylococcus aureus; PVL, Panton-Valentine leukocidin; SCC_mec, Staphylococcal cassette chromosome mec; MLST, multi-locus sequence typing; CA, chloramphenicol; CL, Clindamycin; CIP, Ciprofloxacin; EM, Erythromycin; GM, Gentamicin; RFP, Rifampin; TMP/SMX, Trimethoprim/sulfamethoxazole; TET, Tetracycline; VM, Vancomycin

S, Susceptible; R, Resistant